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The link between relative brain size and cognitive ageing in female guppies (*Poecilia reticulata*) artificially selected for variation in brain size

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ABSTRACT

Cognitive ageing is the general process when certain mental skills gradually deteriorate with age. Across species, there is a pattern of a slower brain structure degradation rate in large-brained species. Hence, having a larger brain might buffer the impact of cognitive ageing and positively affect survival at older age. However, few studies have investigated the link between relative brain size and cognitive ageing at the intraspecific level. In particular, experimental data on how brain size affects brain function also into higher age is largely missing. We used 288 female guppies (*Poecilia reticulata*), artificially selected for large and small relative brain size, to investigate variation in colour discrimination and behavioural flexibility, at 4–6, 12 and 24 months of age. These ages are particularly interesting since they cover the life span from sexual maturation until maximal life length under natural conditions. We found no evidence for a slower cognitive ageing rate in large-brained females in neither initial colour discrimination nor reversal learning. Behavioural flexibility was predicted by large relative brain size in the youngest group, but the effect of brain size disappeared with increasing age. This result suggests that cognitive ageing rate is faster in large-brained female guppies, potentially due to the faster ageing and shorter lifespan in the large-brained selection lines. It also means that cognition levels align across different brain sizes with older age. We conclude that there are cognitive consequences of ageing that vary with relative brain size in advanced learning abilities, whereas fundamental aspects of learning can be maintained throughout the ecologically relevant life span.

1. Introduction

Cognitive ageing is the process of age-related decline of certain cognitive abilities, caused by degradation of brain structures (Hedden and Gabrieli, 2004; Kandel et al., 2013; Marner et al., 2003; Phillips et al., 2018; Ritchie et al., 2015; Scahill et al., 2003). Cognitive ageing is widespread throughout the animal kingdom and studied among diverse species (Behrends et al., 2007; Hirsch and Peretz, 1984; Ritchie et al., 2015; Tapp et al., 2003; Yu et al., 2006; Zwoinska et al., 2013; Zyzak et al., 1995). For example, humans process information at a slower rate and show a severe decline in reasoning and working memory with increasing age (Ritchie et al., 2015), dogs progressively decline in associative learning and behavioural flexibility with increasing age (Tapp et al., 2003), and associative learning and memory declines with increasing age in nematodes (Zwoinska et al., 2013). The causes and consequences of many aspects of cognitive ageing are very well studied in humans and are focused on how to prevent and cure age-related cognitive diseases. However, if evolutionary increases in vertebrate brain size also affect the rate and onset of cognitive ageing is less well understood. Here, we focus on the cognitive consequences of ageing in a laboratory bred guppy population with known differences in brain size.

Learning is an important ability that ranges from simple non-associative learning shared by all bilateral species to more advanced cognitive abilities such as casual reasoning and imagination described in humans, great apes and corvids (Emery and Clayton, 2004; Seed et al., 2009; Shettleworth, 2010). There is ample evidence for the positive effects of learning on survival and reproductive success (Dukas, 2005; Dukas and Bernays, 2000; Dukas and Duan, 2000; Egas and Sabelis, 2001; Grieco et al., 2002; Sherman and Visscher, 2002; Steidle, 1998). Considering the important fitness effects of learning, any decline should

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be disadvantageous. There should thus be strong natural selection for preservation of important aspects of learning ability throughout life time. Within the species tested, the magnitude of cognitive ageing is highly variable, i.e. chronological and physical age can be decoupled. At the proximate level there are both extrinsic and intrinsic sources of variation in cognitive decline. A limited number of studies have investigated why chronological age is not always synchronized with physical age within an ecologically relevant life span in animals. At the intraspecific level, differences in life-history traits, ontogeny and environmental factors have been put forward as important predictors of the onset and rate of cognitive decline. For instance, in honey bees (*A. mellifera*) foragers show a faster decline in olfactory associative learning than nurse bees (Behrends et al., 2007), short-lived female nematodes (*C. remanei*) decline faster than longer-lived males in olfactory associative learning (Zwoinska et al., 2013), and higher water temperature cause faster decline in visual associative learning rate than lower water temperature in the African turquoise killifish (*Nothobranchius furzeri*) (Valenzano et al., 2006). One intrinsic factor that has been suggested to cause variation in cognitive decline is quantitative variation in various aspects of brain size. According to the 'brain reserve' model, higher individual quantitative levels of brain size, neuronal number, number of synapses etc. allow individuals to cope better with brain structure degradation and thereby preserve cognitive abilities into higher age (Katzman, 1993; Satz, 1993; but see Stern, 2009). Hence, a larger 'brain reserve' might delay and slow down cognitive decline compared to a smaller 'brain reserve'. In humans, whole brain volume and/or the volume of certain structures were smaller in individuals with age-related cognitive diseases (Ritchie et al., 2015; Wolf et al., 2004). Theory and empirical findings together imply that quantitative differences in brain morphology might secure brain functionality and thereby preserve cognitive abilities into higher age. However, the link between several aspects of brain morphology and age-related cognitive decline is largely unexplored in non-human animals.

Here, we investigate if ageing has cognitive consequences that vary with quantitative differences in brain size and neuron numbers by means of an experimental approach. We used the reversal learning paradigm to test cognitive performance in three different chronological age groups of female guppies artificially selected for small and large relative brain size, with established substantial differences in brain size (>15%) and neuron number (>11%) (Kotrschal et al., 2013, 2017; Marhounová et al., 2019). Previous experiments on these brain size selected lines have shown that large-brained guppies outperform small-brained guppies in experiments testing various cognitive abilities known to decline with age (Buechel et al., 2018; Kotrschal et al., 2013, 2015a). Since differences in cognitive ability and quantitative measurements of brain morphology is so well established between these up- and down selected lines they emerge as a highly suitable model system to test cognitive performance between different age groups. Based on the 'brain reserve' hypothesis, we expect that with increasing age large-brained females decline in colour association learning ability and behavioural flexibility at a slower rate than small-brained females.

2. Materials and methods

2.1. Experimental design

To examine how relative brain size impacts cognitive ageing, we used individuals with known differences in brain size and neuron number in a cross-sectional design (i.e. individuals from different age groups are compared). We opted to use a cross-sectional design rather than test the same individuals at different ages since the latter might lead to over- or underestimating cognitive ageing if the same test is used multiple times (Harada et al., 2013).

In order to test cognitive ageing, we used the reversal learning paradigm. Reversal learning is a common test of associative learning ability and behavioural flexibility that is frequently used across taxa (Bond et al., 2007; Buechel et al., 2018; Day et al., 1999; Izquierdo et al., 2016; Liu et al., 2016; Sherry and Strang, 2015). During reversal learning, individuals are initially trained to discriminate between two stimuli, rewarded A+ and unrewarded B-. After reaching either a learning criterion or a fixed number of trials, the reward contingency is reversed, i.e. A+ B- becomes A- B+. In order to continue to be rewarded, the animal has to inhibit the previous rewarded response and form a new association. The rate at which individuals learn the new reward contingency has been seen as an indication of behavioural flexibility (Izquierdo et al., 2016). The reversal learning paradigm thus tests for at least two aspects of cognition: the initial part tests for associative learning ability, the reversal part tests for behavioural flexibility.

2.2. Subjects

We used 7th generation female guppies artificially selected for relative brain size (Kotrschal et al., 2013). In short, guppies were up- or down selected for brain weight relative to body length from three independent breeding stocks (replicates), resulting in six lines in total. For more details on the selection regime see Kotrschal et al. (2013). After five generations of selection, a 15.4% difference in relative brain size between up- and down selected lines was established (Marhounová et al., 2019). Recent assays on brain morphology have also shown a difference in brain volume (Kotrschal et al., 2017; Marhounová et al., 2019) and an 11.9% difference in neuron number (Marhounová et al., 2019) between up- and down selected lines. The brains of the fish used in this experiment were not measured directly. Since repeated previous quantification of brain weight, brain volume and neuron number have all shown significant differences in earlier as well as the current generation of selection (we are currently on the 7th generation of selection), we assumed that the previously established differences occur also in the fish used here (Kotrschal et al., 2013, 2017; Marhounová et al., 2019).

We used in total 288 females from three different age groups; 4–6, 12 and 24 months of age, from here on these groups are referred to as the youngest, the middle age and the oldest group. These ages were chosen since they represent the life span from sexual maturation until maximal life length under natural conditions (Reznick et al., 2001). The number of females used were balanced across the three age groups as well as the up- and down selected lines per replicate (i.e. 96 females, 48 small and large-brained, per age group). As all fish from the same generation are bred within a short time range (i.e. three months), the three different age groups were tested at different time points. Only females were used in this experiment since guppy males in general are more difficult to motivate with a food rewards as compared to females due to differences in body size and life history (Houde, 1997; Fuss and Witte, 2019; Kotrschal et al., 2013).

Fish were kept with constant aeration, in 25 ± 1 °C water temperature, on a 12:12 h dark: light cycle, java moss (*Taxiphyllum sp.*) and water snails (*Planorbis sp.*) to facilitate the elimination of organic waste. Fish were fed six days per week with flake food and live *Artemia nauplii*.

2.3. Apparatus

All females were kept individually in 7 L experimental tanks that were divided into a home chamber and a testing chamber (Fig. 1). These were separated by a transparent and a grey sliding door. The grey door prevented the females from perceiving any cues when the experimenter prepared each new trial. The transparent door allowed the females to assess the arrangement in the conditioning chamber prior to each trial. A trial started with opening of the grey door and 10 s later the transparent door was opened. The females had visual contact with each other between the home chambers, to minimize potential negative effects of social isolation (Lombardi-Brandão et al., 2015; Petrazzini et al., 2012), but were prevented from contact between testing chambers to avoid social learning (Brown and Laland, 2002; Reader et al., 2003). A white plate with 20 equispaced wells (10 mm in diameter, 5 mm deep) was

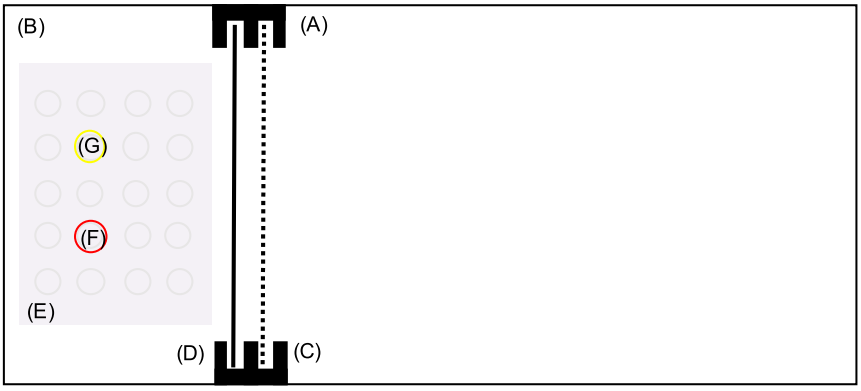

Fig. 1. Schematic diagram of the experimental set-up. All fish were housed individually in experimental tanks throughout the whole experiment. The tanks consisted of a home chamber (A) and a testing chamber (B). These were separated by a transparent (C) and a grey (D) sliding door. A white plate (E) with holes was placed at the bottom in the testing chamber. Fish were trained to discriminate between a red (F) and a yellow plastic disc (G) and to find a frozen and thawed *Artemia* reward underneath the rewarded stimulus colour. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

placed at the bottom in the testing chamber. Prior to the cognitive assays all females were pre-trained to dislodge a black plastic disc (14 mm in diameter, 2 mm thick) placed adjacent to the mid well on the white plate and find an adult frozen and thawed *Artemia* reward underneath. During the first trials of the pre-training, the disc only partly covered the well. During consecutive trials we successively covered the whole well. All females learnt to dislodge the black disc within 30 trials. Moving an object to find food underneath is part of the behavioural repertoire of guppies since under natural conditions guppies are known to forage underneath plant parts etc. (Houde, 1997). The experimenter was blind to the selection line of all females in order to avoid any potential observer bias.

2.4. Cognitive assay

Initially, we trained the females in a binary colour discrimination task. We used, red and yellow colour stimuli, in consideration of the ecology of female guppies. Orange colours are important cues during mate choice and foraging (Houde, 1997; Rodd et al., 2002). Red and yellow have also successfully been used in similar experiments using female guppies (Buechel et al., 2018; Fong et al., 2019; Lucon-Xiccato and Bisazza, 2014). To control for potential colour preferences, the rewarded stimuli were balanced for each age group across the up- and down selected lines per replicate. That is, half of all small and large-brained females respectively learnt to associate yellow with the *Artemia* reward and the other half learnt to associate red with the *Artemia* reward. To ensure that the females learnt to discriminate between the stimuli, the position of the rewarded stimulus, right vs left, was haphazardly chosen by rolling a dice, but making sure that no more than two consecutive trials were performed in the same position. The rewarded stimulus (S+) was free to slide from the well, whereas the unrewarded stimulus (S-) was unmovable due to a glued-on silicon knob that protruded down into the well. To control for odour effects, an *Artemia* reward was placed under both the rewarded and the unrewarded stimulus. For each trial, we scored the first push a female did on either of the disc as a correct (1) or incorrect (0) response. If the female did not push any of the discs within 120 s, that trial was recorded as a non-choice trial, but we allowed the female to solve the task to provide all females with the same number of positively reinforced trials. The females were given one daily six-trial session during four days, i.e. 24 trials in total.

To test behavioural flexibility, the reward contingencies were reversed following the fixed number of trials (i.e. 24) in the initial colour discrimination, i.e. S+ became S- and vice versa. The training protocol was equivalent to the protocol described in the initial colour discrimination, but the females were given seven days of daily six-trial sessions, i.e. 42 trials in total. Three individuals, one large-brained from the middle age group and two small-brained from the oldest group, were excluded from the reversal learning part due to acclimatization problems to the new reward contingency (after making several errors during the first reversal trials they showed high stress levels and were removed in accordance with our ethical permits).

2.5. Data analysis

Statistical analyses were performed using R statistical software (v 3.5.1, http://R-project.org/). To assess the impact of relative brain size on learning rate and flexibility between three different age groups, we used generalized linear mixed effects models (GLMMs) with binomial error distribution (1 = correct response, 0 = incorrect response) and logit link functions, as implemented with the *glmer* function in the *lme4* package (Bates et al., 2014). We fitted separate models for the initial colour discrimination and the reversal learning since they test different aspects of cognition. More specifically, for each cognitive assay and age group we modelled correct/incorrect response as a function of the fixed effects brain size (small, large), trial number, rewarded colour (red, yellow), brain size × trial and brain size × rewarded colour, were the brain size × trial interaction test for differences in slope (i.e. learning rate) between small and large-brained females and the brain size × rewarded colour interaction test for differences in slopes between small and large-brained females depending on rewarded colour. Since the reversal learning part is strongly affected by the performance in the colour discrimination, and we detected differences in colour discrimination across the three different age groups (see results, Section 3.1.), we fitted separate models for the three different age groups. To account for repeated measurements, the random effect fish ID was included. Since it is biologically reasonable to expect each unique individual to learn differently from other individuals, we fitted individual learning curves for each fish, i.e. random intercept and slope (Schielzeth and Forstmeier, 2009). In all initial full models, we fitted a random intercept for brain size nested in replicate. Replicate returned a zero-variance estimate. This zero-variance caused singular fit to the models. To still control for replicate we thus fitted it as a fixed factor, but dropped it from further analyses since it was non-significant ($p > 0.4$ for all models) and inclusion of replicate did not improve model fit ($\Delta AIC > 2$ for all models). [*glmer syntax* for all full models: correct/incorrect response ~ brain size + trial + rewarded colour + brain size × trial + brain size × rewarded colour + (trial | fish ID)].

The continuous predictor variable 'trial' was standardized to zero mean prior to running models. To ensure model convergence, we used the optimizer *bobyqa* (*Nelder_Mead* was used for the model testing initial colour discrimination in the middle age group) and increased the number of iterations, as implemented with the *glmerControl* function. Model selection was done backwards based on Akaike's information criterion, only non-significant interactions were subject to any exclusion (Bolker, 2008). Statistical significance was obtained by using the ANOVA function, specifying Type III Wald chi-square tests, in the *car* package (Fox and Weisberg, 2019).

3. Results

3.1. Initial colour discrimination

We found that relative brain size did not predict colour discrimination learning rate or the probability of average correct responses in any of the three age groups (Table 1). Small and large-brained females learnt to discriminate between red and yellow at equal rate in the youngest, middle aged and oldest group (Fig. 2). Importantly, trial number predicted correct responses (Table 1), suggesting that all females learnt the initial discrimination task (Fig. 2). Rewarded colour predicted the probability of correct responses (Table 1). Females trained on red responded correctly more often than females trained on yellow in the youngest (raw data mean ± s.e. 94.0 ± 0.07% correct responses for females trained on red versus 74.8 ± 1.3% correct responses for females trained on yellow) and the middle age groups (raw data mean ± s.e. 87.1 ± 1.0% correct responses for females trained on red versus 75.9 ± 1.3% correct responses for females trained on yellow). In the oldest group, females trained on yellow responded correctly more often than females trained on red (raw data mean ± s.e. 91.2 ± 0.8% correct responses for females trained on yellow versus 84.0 ± 1.1% correct responses for females trained on red).

During the last six trials, the youngest and oldest groups reached similar mean performance levels while the middle age group reached a slightly lower level (raw data mean ± s.e. 93.5 ± 1.0%$_{youngest}$, 90 ± 1.3%$_{middle}$ and 94.1 ± 1%$_{oldest}$).

3.2. Behavioural flexibility

We found that relative brain size predicted behavioural flexibility in the youngest group, such that large-brained females learnt the new reward contingency at a faster rate and made more correct choices (Table 2; Fig. 3). Brain size interacted with colour, indicating that large-brained females had a preference for red in the youngest age group (Table 2). Behavioural flexibility was not predicted by relative brain size in the middle age group (Table 2; Fig. 3). The model revealed a significant main effect of trial, suggesting that all females learnt the new reward contingency, and also for rewarded colour, suggesting that red was a more salient stimulus (Table 2). In the oldest group, behavioural flexibility was not predicted by relative brain size. Small and large-brained females learnt the new reward contingency at equal rate and did equal amount of correct responses (Table 2, Fig. 3). The model also revealed significant effects of trial and rewarded colour, suggesting that all females learnt the new reward contingency and that females trained on yellow did more correct responses (Table 2).

Overall, the three different age groups reached similar mean performance levels the last six trials (raw data mean ± s.e. 87 ± 1.4%$_{youngest}$, 88 ± 1.4%$_{middle}$ and 87 ± 1.4%$_{oldest}$).

Table 1
Results from independent GLMMs testing for the effect of relative brain size on initial colour discrimination ability across three different age groups of female guppies. The columns provide chi-squared values (χ^2), degrees of freedom (d.f.) and associated significance values (p) for the fixed effects brain size (small, large), trial number (1–24) and rewarded colour (red, yellow). Significant ($p < 0.05$) effects are highlighted in bold. We also report the logistic regression slope estimates and their standard errors (SE). Brain size small, mid trial (see methods, Section 2.5.) and colour red are set as baseline.

Fixed effects	χ^2	d.f.	p - Value	Estimate ± SE
Youngest group (4–6 months old)				
(Intercept)	245.48	1	<0.001	3.08 (0.20)
Brain size	0.60	1	0.44	0.14 (0.18)
Trial	99.67	1	**<0.001**	0.14 (0.01)
Colour	90.43	1	**<0.001**	−1.87 (0.20)
Middle age (12 months old)				
(Intercept)	114.05	1	<0.001	2.23 (0.21)
Brain size	0.36	1	0.55	0.14 (0.23)
Trial	58.78	1	**<0.001**	0.10 (0.01)
Colour	15.78	1	**<0.001**	−0.91 (0.23)
Oldest group (24 months old)				
(Intercept)	93.91	1	<0.001	2.08 (0.22)
Brain size	0.05	1	0.82	0.05 (0.24)
Trial	52.10	1	**<0.001**	0.13 (0.02)
Colour	12.14	1	**<0.001**	0.86 (0.25)

4. Discussion

The onset and rate of cognitive ageing has been proposed to be affected by individual differences in brain size and neuron number. To test this, we investigated cognitive performance in female guppies from three different age groups artificially selected for small and large relative brain size. We found that a relative larger brain with more neurons did not preserve cognitive abilities into higher age better than a smaller brain as predicted by the 'brain reserve' hypothesis. In contrast, the evident positive effect of relative brain size on behavioural flexibility in the youngest age group disappeared with increasing age. Initial colour discrimination learning rate did not vary with relative brain size across age in these selection lines. Interestingly, we found no obvious effect of age neither during the initial colour discrimination nor during the reversal learning in these female guppies.

In line with a previous study (Buechel et al., 2018), we found a significant interaction between brain size and trial (i.e. differences in slopes and thus learning rate) during reversal learning in the youngest age group, but this effect was not evident in the middle and the oldest age groups. We interpret this as a relative decline in behavioural flexibility in large-brained female guppies. Our results might thus support an evolutionary trade-off between relative brain size and maintenance of advanced cognitive abilities into higher age. One explanation for such a trade-off between relative brain size and cognitive ageing is how resources are allocated differently depending on life-history strategy. Life-history theory predicts that high investment in important fitness-related traits early in life will be traded-off against a faster senescence and a shorter life span. While a longer life span would maintain these traits into higher age (Kirkwood and Holliday, 1979; Williams, 1957). Most notably in this context, a recent comparison in longevity between the small and large-brained guppies revealed a 22% shorter intrinsic life span in the large-brained guppies (Kotrschal et al., 2019). Hence, any cognitive benefits early in life of having a larger, more neuron rich brain, might be lost later in life if general and cognitive ageing occurs faster. The alignment in cognitive levels during reversal learning found here is consistent with other findings in animals with natural or artificial dimorphic life spans. The short-lived sex in nematodes (*C. remanei*) outperformed the long-lived sex in a cognitive assay early in life, but showed a faster cognitive ageing rate (Zwoinska et al., 2013). Artificial selection for improved learning abilities in fruit flies (*Drosophila melanogaster*) reduced life span with 15% in up-selected lines compared to controls (Burger et al., 2008). Similar patterns have been found in long-lived mutants of nematodes (Murakami et al., 2005) and mice (Bartke, 2005). Taken together, this supports a general pattern where cognitive ageing rate appears to be faster in short-lived animals compared to long-lived conspecifics. Furthermore, our results strengthen previous results by Kotrschal et al. (2013, 2019), that evolutionary changes in relative brain size cause changes in life-history strategy also at the intraspecific level. The faster learning rate during reversal learning found in young large-brained females indicate that they allocate more resources into cognition and presumably into the development and maintenance of brain tissue early in life. In contrast, small-brained females appear to allocate more resources into somatic maintenance (Kotrschal et al., 2013, 2016). A higher cognitive ability early in life might increase extrinsic survival and thereby secure future reproductions.

Our results do not support the 'brain reserve' hypothesis. One potential explanation for this could be that the hypothesis predicts patterns in cognitive ageing rate mainly at the interspecific level. Across taxa,

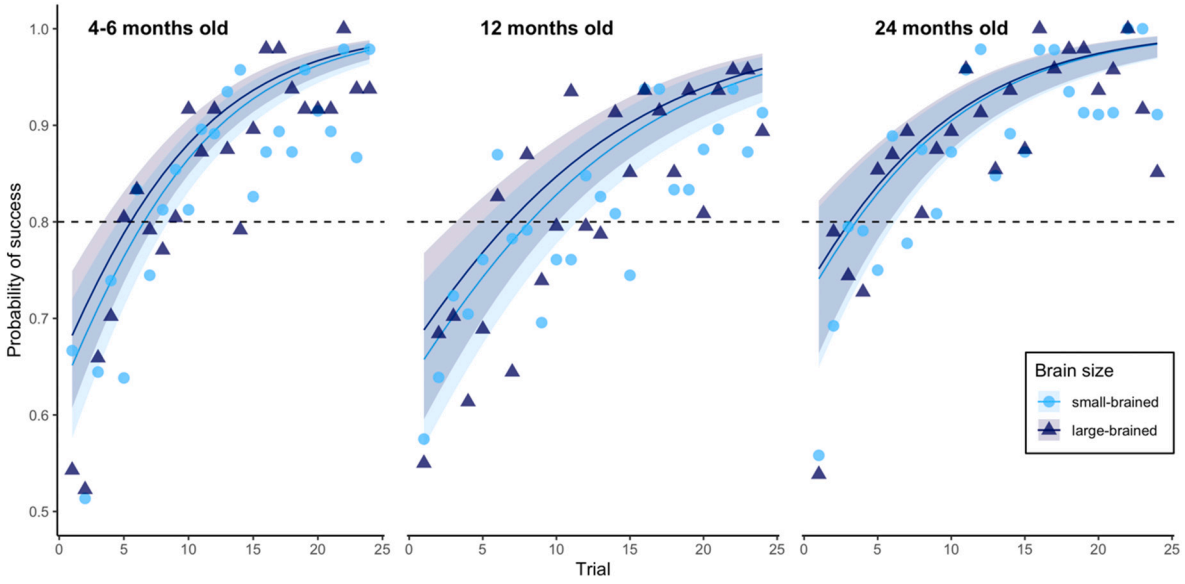

Fig. 2. Initial colour discrimination. Proportion of correct responses each trial in a binary colour discrimination task in 288 female guppies of three different age groups. Circles, clear blue line and 95% CI signify raw data means and GLMM predictions for small-brained females; diamonds, dark blue line and 95% CI signify raw data means and GLMM predictions for large-brained females. Means and model predictions were established from 6912 observations in total across 24 trials. The dotted line represents 80% correct responses level. We found no difference in colour discrimination learning rate between small and large-brained female that changed with increasing age. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Results from independent GLMMs testing for the effect of relative brain size on behavioural flexibility across three different age groups of female guppies. The columns provide chi-squared values (χ^2), degrees of freedom (d.f.) and associated significance values (p) for the fixed effects brain size (small, large), trial number (1–42) and rewarded colour (red, yellow). Significant ($p < 0.05$) effects are highlighted in bold. We also report the logistic regression slope estimates and their standard errors (SE). Brain size small, mid trial (see methods, Section 2.5.) and colour red are set as baseline.

Fixed effects	χ^2	d.f.	p - Value	Estimate ± SE
Youngest group (4–6 months old)				
(Intercept)	37.12	1	<0.001	1.10 (0.18)
Brain size	4.23	1	**0.04**	0.53 (0.26)
Trial	158.83	1	**<0.001**	0.10 (0.01)
Colour	0.04	1	0.83	0.05 (0.22)
Brain size × trial	5.17	1	**0.02**	0.02 (0.01)
Brain size × colour	5.35	1	**0.02**	−0.73 (0.32)
Middle age (12 months old)				
(Intercept)	112.54	1	<0.001	1.51 (0.14)
Brain size	1.61	1	0.20	0.18 (0.14)
Trial	270.76	1	**<0.001**	0.10 (0.01)
Colour	14.80	1	**<0.001**	−0.55 (0.14)
Oldest group (24 months old)				
(Intercept)	21.84	1	<0.001	0.74 (0.16)
Brain size	0.68	1	0.41	0.15 (0.18)
Trial	380.61	1	**<0.001**	0.11 (0.01)
Colour	4.50	1	**0.03**	0.38 (0.18)

absolute and relative brain size have (with a few exceptions) increased considerably during vertebrate evolution (Jerison, 1973; Striedter, 2006; Tsuboi et al., 2018). At the interspecific level, species with a larger brain than expected for their body mass generally have a longer life span (Allman et al., 1993; Barrickman et al., 2008; González-Lagos et al., 2010; Isler and van Schaik, 2009; Jiménez-Ortega et al., 2020; Sol et al., 2007, 2016; Yu et al., 2018). However, this pattern is apparently often reversed at the intraspecific level. For instance, large-brained guppies live significantly shorter than their small-brained conspecifics (Kotrschal et al., 2019). As discussed above, a longer life span often correlates with a slower cognitive ageing rate within several species (Bartke, 2005; Burger et al., 2008; Murakami et al., 2005; Zwoinska et al., 2013). Therefore, we speculate that quantitative differences in brain anatomy, together with general differences in life-history and ecology, explain cognitive ageing rate in accordance with the 'brain reserve' hypothesis at the macroevolutionary level, but not at the intraspecific level. The slower brain degradation in large-brained compared to small-brained species corroborates this speculation (Vágási et al., 2016). Interestingly, the patterns in humans makes for a striking exception to this pattern. There are several possible explanations to this pattern. First, humans exceed their expected brain size for their body weight more than any other vertebrate (Jerison, 1973; Striedter, 2006; Tsuboi et al., 2018). Second, together with the increase in brain size, humans have also extended their life span more than other primates (Allen et al., 2005; Allman et al., 1993). Third, grandmaternal care during post-reproductive life stages may increase inclusive fitness and select for preservation of cognitive abilities into higher age (Allen et al., 2005).

It is clear that also in non-human animals cognitive ageing is a not a general process that causes decline in all aspects of cognition. Rather, cognitive ageing causes decline in certain independent modules of cognitive abilities (Bartus et al., 1979; Hedden and Gabrieli, 2004; Izquierdo et al., 2016; Lai et al., 1995; Voytko et al., 1999). In support of this, we found no obvious decrease in initial colour discrimination learning rate as the mean performance the last six trials was similar across the three different age groups. This suggests that these simpler cognitive abilities are maintained throughout the ecologically relevant life span in guppies that this study encompassed. Also, red and yellow colours are biologically important cues for female guppies (Houde, 1997; Rodd et al., 2002). Selection might therefore be strong to preserve these traits also into higher age. This finding contrasts with what has been found in invertebrates (nematodes: Zwoinska et al., 2013; and honey bees: Behrends et al., 2007) and in the African turquoise killifish (Valenzano et al., 2006), but is supported by findings in rhesus monkeys (Bartus et al., 1979; Lai et al., 1995), all tested within their ecologically relevant life span. Interestingly, behavioural flexibility was also mainly maintained through increased age in our guppy selection lines, as we found no substantial decrease in mean performance the last six trials across the three different age groups. This suggests that, despite the alignment between small and large-brained females with increasing age,

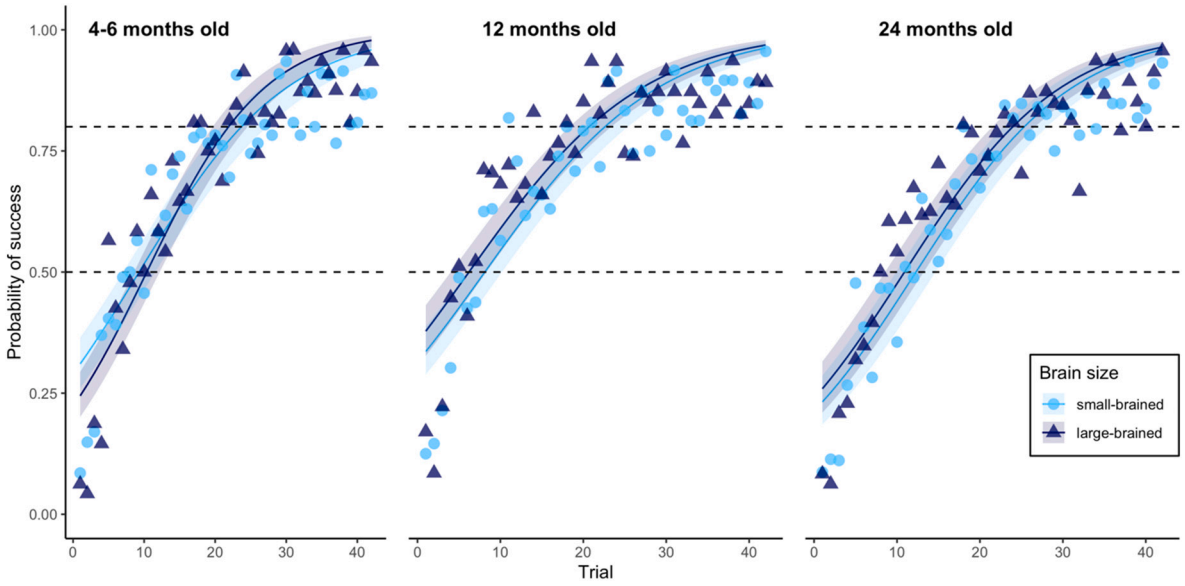

Fig. 3. Reversal learning. Proportion of correct responses each trial in a colour reversal learning task in small and large-brained female guppies of three different age groups. Circles, clear blue line and 95% CI signify raw data means and GLMM predictions for small-brained females; diamonds, dark blue line and 95% CI signify raw data means and GLMM predictions for large-brained females. Means and model predictions were established from 12,054 observations in total across all 42 trials. The dotted line represents 50% and 80% correct responses level. We found that learning rate and correct responses were predicted by relative brain size in the youngest group (brain size × trial; $\chi^2_1 = 5.17$, $p = 0.02$: brain size; $\chi^2_1 = 4.23$, $p = 0.04$). In the middle age and the oldest group neither learning rate nor correct responses were predicted by relative brain size. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

also more complex cognitive abilities are largely maintained across the ecologically relevant life span in female guppies. Also, this result contrasts with findings in rhesus monkeys, where behavioural flexibility declined with increasing age (Bartus et al., 1979; Lai et al., 1995; Voytko, 1999). Since cognitive decline is caused by degradation in brain structures (Finkel and Holbrook, 2000; Hedden and Gabrieli, 2004; Marner et al., 2003; Phillips et al., 2018), the current and earlier findings imply that there might be interspecific variation in the level of degradation of brain sub-structures. These differences might in turn be caused by interspecific differences of the importance of a particular cognitive trait (Bond et al., 2007; Day et al., 1999; Sherry and Strang, 2015), also in higher age. We speculate that the social complexity of a species might partly explain these differences. For instance, in long-lived group living species with a heterogeneous age structure, the decline in flexibility in older individuals might be compensated by high levels of behavioural flexibility in younger individuals. Hence, selection pressure for preservation of a given cognitive ability might be low. In solitary species or in species with a homogeneous age structures, selection pressures for preservation of various cognitive abilities into higher age might be stronger. Guppies shoal in relatively homogeneous age groups in wild populations (Croft et al., 2003), which might thus explain why we did not find any substantial overall decrease with increasing age in the cognitive abilities tested here. We speculate that social complexity can be a potentially important factor that cause interspecific variation in cognitive ageing rate. However, while this 'sociality allows for cognitive ageing' hypothesis might be relevant for animal species that form groups with heterogeneous age structures remains to be tested.

During the initial colour discrimination, red was a more salient stimulus for the youngest and the middle age groups. Whereas yellow was instead a more salient stimulus for the oldest group. A pre-existing bias for red colours are well known in female guppies, as it is an important signal for male quality and nutrient food resources (Houde, 1997; Rodd et al., 2002). Experience is known to affect pre-existing biases (Hebets, 2003; Svensson et al., 2010; Westerman et al., 2012). However, these guppies are bred under constant laboratory conditions and it is unlikely that this shift is caused by differences in experience between the groups. Alternatively, there might be age-related physiological processes that causes a shift in the salience of a signal. Shift in pre-existing biases and preferences during mate choice have been found to vary with age in both female guppies (Kodric-Brown and Nicoletto, 2001) and female wolf-spiders (FowlerFox et al., 2015; Uetz and Norton, 2007). Therefore, we speculate that this shift in salience is caused by age-related physiological processes rather than confounding environmental conditions. Future tests of visual acuity and colour vision across differently aged guppies are necessary to address this question.

5. Conclusions

Overall, our results show that cognitive ageing is indeed a complex process, generated by a wide array of intrinsic and extrinsic factors that most likely cause extensive variation at all taxonomic levels in the affected neural networks. We conclude that the 'brain reserve' hypothesis does not fully explain the relationship between relative brain size and cognitive ageing at the intraspecific level. Instead, our results indicate that evolving a larger brain might generate important cognitive advantages in certain contexts early in life, but a slightly faster cognitive ageing rate. In addition to the many benefits of evolving a larger brain (Kotrschal et al., 2013, 2015b; MacLean et al., 2014; Sol et al., 2007; van der Bijl et al., 2015), there are also substantial costs associated with increased brain size (Kotrschal et al., 2013, 2016; Raichle and Gusnard, 2002; Tsuboi et al., 2015). It has also previously been shown that there is a negative relationship between relative brain size and life span at the intraspecific level (Kotrschal et al., 2019). We propose that a faster cognitive ageing rate is caused by the shorter life span generated by a larger relative brain size at the intraspecific level and suggest that this can be yet another aspect that constrain the evolution of increased brain size.

Ethics

The experiment was performed in accordance with ethical applications approved by the Stockholm Animal Research Ethical Permit Board (Dnr: N173/13, 223/15, N8/17 and 17362-2019).

CRediT authorship contribution statement

Conceptualization: A.B., N.K.
Methodology: A.B., A.K., N.K.
Formal analysis: A.B.
Investigation: A.B.
Resources: N.K.
Writing original draft: A.B.
Writing review and editing: A.B., S.B., M.A., A.K., N.K.
Visualization: A.B.
Supervision: N.K.
Project administration: S.B., M.A.
Funding acquisition: N.K.

Declaration of competing interest

The authors have no conflicts of interest.

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